PROCESS DEVELOPMENT FOR BACTERIORHODOPSIN EXPRESSED IN *Escherichia coli*

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ABSTRACT

The Army is increasingly dependent on computers and electronic tools to achieve high levels of situational awareness on the battlefield (Opportunities in Biotechnology for Future Army Applications, National Research Council, 2001). With silicon based technologies reaching their technological limits, biologically derived or inspired materials such as protein-based memory systems are viable alternate technologies for the future which will increase their usefulness for Army applications. Bacteriorhodopsin, a light-activated protein with unique photophysical properties, an excellent candidate which can be exploited for volumetric data storage, provides promising new methodologies for high-speed signal processing and communication, novel neural networks, linear and nonlinear devices and memories, which can revolutionize the Army's strategic communication infrastructure. Large quantities of readily available bacteriorhodopsin would be especially useful for the further developing bio-derived electronic and photonic materials. In this manuscript we describe the optimization of production and purification protocols for bacteriorhodopsin recovery and yield from a recombinant *E. coli* fermentation system.

1. INTRODUCTION

Bacteriorhodopsin (bR), an integral membrane protein with seven transmembrane domains in its single polypeptide chain, is the light transducing protein in the purple membrane of *Halobacterium halobium*. The intrinsic properties of bR, such as long-term stability of the protein to thermal and photochemical degradation, wavelength-independent quantum yields, and the ability to form thin films with excellent optical properties, make it an outstanding candidate for use in an optically coupled device (optical switches, holographic and volumetric 3-D memories for information storage), spatial light modulation, nonlinear optical filters and pattern-recognition systems. The Army will have a growing need for computer memory capacity in the battlefield to support the increasing use of graphical formats to facilitate the assimilation of information in real time, (Opportunities in Biotechnology for Future Army Applications, National Research Council, 2001).

The recent interest in the enormous application potential of bR, led us to develop an enhanced recombinant protein production and purification method using *E. coli* fermentations. The low abundance of this membrane protein and it's hydrophobic nature make it difficult to isolate and purify in amounts required for structural and functional applications. One of the biggest challenges is obtaining integral membrane proteins from inclusion bodies in the cytoplasm of the bacteria in a functional, solubilized, and monodisperse state that provides a native-like environment that maintains the spectrum of *in vivo* activities. The *in vitro* construction of an affinity tag system facilitates the expression and downstream bioprocessing of recombinant proteins from endogenous cellular substances. The objective of this project is to develop a scaleable and reproducible recombinant protein production and downstream/ purification protocol for bR expressed in *E. coli*.

2. RESULTS

A bacteriorhodopsin gene from *Halobacterium* (ATCC 700922) was cloned into plasmid pET21b and expressed in *E. coli* strain BL21(DE3). The bR insert was verified by agarose gel electrophoresis. The presence of bR in the *E. coli* was verified by SDS-PAGE and Western blot. The histidine-tagged protein was produced in 5L fermentors using transformed *E. coli* BL21(DE3). A typical 5L fermentation profile of *E. coli* showing the dissolved oxygen (DO) concentration and growth is given in Figure 1.

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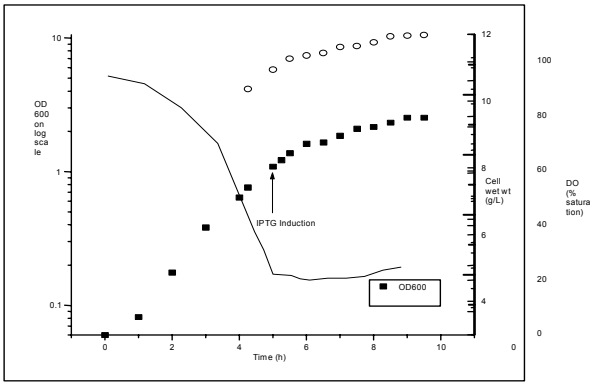

Figure 1. Fermentation profile of *E. coli* showing DO demand and growth

The expressed protein was localized primarily to the *E. coli* cytoplasmic membrane as an inclusion body and the isolation and purification required cell lysis and solubilization of the protein by replacement of the lipids by detergents. Different cell lysis procedures were compared for optimal protein yield. Crude cell lysate of *E. coli* expressing $(His)_6$ - tagged bR was efficiently purified by Immobilized Metal Affinity Chromatography (IMAC) using different metal ions (Ni^{2+}, Co^{2+}) under denaturing conditions, analyzed by High Performance Liquid Chromatography (HPLC) and the expression levels were quantified. The charge and size homogeneity of the obtained detergent-protein complex was analyzed by ion exchange and analytical size exclusion chromatography. An expression level of 65 mg/L at an approximate purity level of 85% was obtained. Membrane proteins in detergent solution frequently tend to aggregate or become denatured. Optimization studies on purification protocol, renaturation and subsequent reconstitution of the bR into vesicles, and the functional properties of the protein will be described.

3. SUMMARY

In summary, these experiments have shown that bacteriorhodopsin can be expressed in *E. coli*. The results show that optimizing the fermentation and downstream/ purification process can result in significant improvements in the protein recovery and yield.

REFERENCE

Opportunities in Biotechnology for Future Army Applications (2001), Board on Army Science and Technology, National Research Council, National Academy Press, Washington, D.C.